

Chronic bovine besnoitiosis: histopathological findings and parasite distribution and load in subclinical cases

Frey C.F.^{1,2}, Gutiérrez-Expósito D.¹, Ortega-Mora L.M.¹, Benavides J.³, Marcén J.M.⁴, Castillo J.A.⁴, Casasús I.⁵, Sanz A.⁵, García-Lunar P.¹, **Álvarez-García G.^{1*}**

1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University;

2 Institute of Parasitology, Vetsuisse Faculty, Univ. of Bern, CH-3001 Bern, Switzerland;

3 Departamento de Patología Animal, Medicina Animal (Anatomía Patológica), Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain;

4 Animal Pathology Department, Faculty of Veterinary Sciences, University of Zaragoza, Miguel Servet 177, 50013-Zaragoza, Spain;

5 Unidad de Tecnología en Producción Animal, Centro de Investigación y Tecnología Agroalimentaria - Gobierno de Aragón, Avda. Montañana 930, 50059 Zaragoza, Spain.

*email: gemaga@vet.ucm.es. Tel. +34 913944095.

Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is a chronic and debilitating disease. The most characteristic clinical signs of chronic besnoitiosis are visible tissue cysts in the scleral conjunctiva and the vagina, thickened skin, and a generally poor body condition. However, many seropositive animals remain subclinically infected, and the role that these animals play in spreading the disease is not known. The aim of the present study was to assess the serological status, tissue distribution, and parasite load of subclinically infected animals. Histopathological, immunohistochemical and molecular analyses were performed using several tissues from the respiratory and reproductive systems, in addition to other internal organs and skin, from six cows that had exhibited scleral cysts and specific antibodies in the past but did not show any clinical signs at the time of slaughter. Tissue cysts were located primarily in the upper respiratory tract, i.e., the rhinarium and larynx/pharynx, were found in 4 cows. The next most common cyst locations were the distal genital tract (vulva/vagina) and the skin of the neck, found in 3 and 2 cows, respectively, out of the 4 cows showing cysts in the respiratory tract. We were unable to detect any parasite in the remaining 2 cows. Tissue cysts were located in the conjunctive tissue, and in two cows, these cysts were associated with a non-purulent inflammatory infiltrate consisting primarily of T lymphocytes. The correlation between the histopathological results and PCR was very good, although the latter was moderately more sensitive. The parasite burden, estimated based on the number of cysts and the quantitative real time-PCR results, was very low. It is noteworthy that the only animal that showed a recent increase in seropositivity showed the highest burden and the most conspicuous inflammatory reaction against the cysts.

In conclusion, although these cows no longer displayed any visible signs of besnoitiosis, they remained infected and therefore may still be able to transmit the parasite.